

## CHROMOSOMES OF SIXTEEN SPECIES OF HARVESTMEN (ARACHNIDA, OPILIONES, CADDIDAE AND PHALANGIIDAE)

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### ABSTRACT

Chromosomes of *Caddo agilis* (Caddidae) and fifteen species of Phalangiidae were investigated. In three species, *Nelima satoi*, *N. similis*, and *Eumesosoma roeweri*, presence of XY-XX (male heterogametic) sex chromosome system was newly ascertained. On the other hand, ZW-ZZ (female heterogametic) sex chromosome system was suggested to be present in *Mitopus morio*. Effeminate ( $2n = 20$ ) and normal ( $2n = 18$ ) males of *Protolophus tuberculatus* were found to differ in chromosome number. A survey of known records of chromosome numbers in Caddidae and Phalangiidae revealed a general trend that the number is greater in both Caddidae ( $2n = 30$ ) and Phalangiinae ( $2n = 20-36$ ), fewer in Gagrellinae ( $2n = 10-22$ ), and intermediate in Leiobuninae ( $2n = 16-26$ ). Evolutionary trends are briefly discussed and compared with those in other arachnids.

### INTRODUCTION

Studies on chromosomes of harvestmen are few with the counts of only 36 species being reported (Tsurusaki 1986). Chromosomal observation has, however, great importance in gaining comprehensive understanding of geographic variation, speciation process, and phylogeny of Opiliones, since chromosomes of harvestmen often vary among related species and sometimes among geographical populations within the same species (e.g., genus *Leiobunum*: Suzuki 1976a; Tsurusaki 1985a, b).

To advance our general knowledge of opilionid chromosomes, we have prepared chromosome slides over the past seven years. This paper is the result of this study and describes chromosomes of fifteen species of Phalangiidae and one species of Caddidae.

### MATERIALS AND METHODS

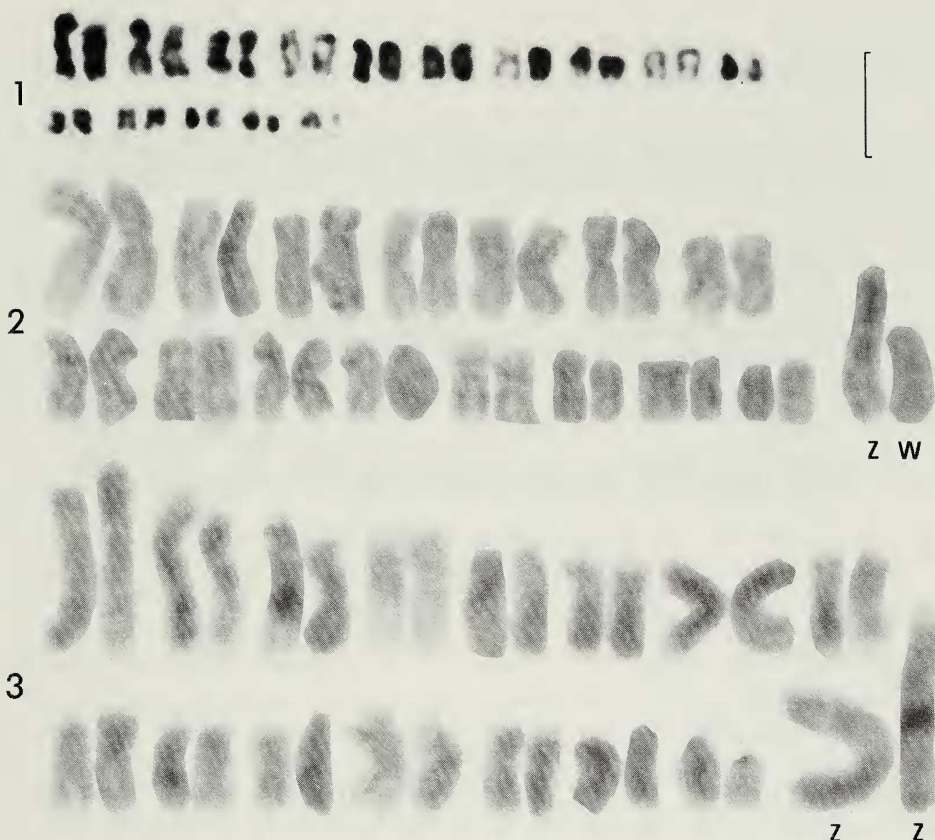
Sources of the specimens are listed in Table 1 and in the appendix. Chromosome preparations were prepared from testes or ovarian tissues of young adults and penultimates. Air-dried slides were made principally according to the method described in Tsurusaki (1985a) for the species from Japan and the

Table 1.—A list of materials used in the present study and obtained results. Detailed collecting data are given in the Appendix. M = male(s), F = female(s), juv. = juvenile(s). 2n chromosome number in parentheses denotes value inferred from haploid number alone. For distinction of the geographic forms of *Melanopa grandis*, see text.

Species	Locality	No. indiv. obs.	2n chrom. number		No. modal cell (M/F)
			M	F	
<i>Caddo agilis</i>	HOKKAIDO:Nopporo	9 juv. (F)	—	30	41
<i>Mitopus morio</i>	Is. Rishiri	3 juv.(2M, 1F)	32	32	9/3
<i>Homolophus arcticus</i>	HOKKAIDO: Wakasakanai	3 juv. (M)	24	—	25
<i>Homolophus rishiri</i>	Is. Rishiri	2 juv. (M)	24	—	13
<i>Phalangium opilio</i>	IDAHO:Moscow	1 M	32	—	1
<i>Dalquestia formosa</i>	TEXAS:Center Point	1 M	22	—	1
<i>Nelima satoi</i>	EHIME: Mt. Ishizuchi	2 juv. (F)	—	16	2
	FUKUOKA: Mt. Hiko	1 juv. (M)	16	—	11
<i>Nelima similis</i>	NAGANO:Takatô	4 M	20	—	11
<i>Leiobunum flavum</i>	TEXAS:L.Stubblefield	4 M	22	—	12
<i>Leiobunum townsendi</i>	TEXAS:Concho Co.	1 M	20	—	1
<i>Eumesosoma roeweri</i>	TEXAS:Concho Co.	2 M, 2F	22	22	3/2
	TEXAS:Kerrville	1 M	22	—	4
<i>Protolophus tuberculatus</i>	CALIFORNIA:San Anselmo	1M (1983)	(20)	—	5
	CALIFORNIA:San Anselmo	1 M (1984)	18	—	8
<i>Protolophus</i> sp.	CALIFORNIA:Little Sycamore Canyon	2 M	22	—	1
<i>Trachyrhinus rectipalpus</i>	TEXAS:Tilden	1 M	10	—	3
<i>Melanopa grandis</i>	Form 1 NAGANO:L.Misuzu	1 juv. (M)	20	—	5
	Form 1 NAGANO:Mt. Kirigamine	1 juv. (M)	20	—	5
	Form 2 TOTTORI:Mt. Daisen	3 M	20	—	6
	Form 3 FUKUOKA: Mt. Hiko	3 M	20	—	25
	Form 3 Is. Tsushima: Hidakatsu	1 M	20	—	16
	Form 1 Is. Tsushima: Mt. Ariake	1 M	20	—	8
<i>Paraumbogrella pumilio</i>	HOKKAIDO:Sunagawa	1 M	10	—	4

method in Cokendolpher and Brown (1985) for the species from the U.S.A. Of these methods, the procedures of the former were slightly modified in preparations after 1984 as follows: (1) use of Ringer's solution at the first step was abandoned and specimens were directly dissected in hypotonic solution. The tissues were removed and transferred to the same solution on another depression slide for hypotonic treatment; (2) as the hypotonic solution, 0.1% sodium citrate with colchicine (19 parts of 1% sodium citrate to one part of 0.1% colchicine solution) was used instead of pure 1% sodium citrate.

Metaphase chromosomes were serially arranged according to descending order of length (Figs. 1-3, 8-11, 18-24, 29-33). When a pair of heteromorphic chromosomes were observed only in either sex, those were considered as sex chromosomes. Haploid idiograms of each species were drawn based on a somatic metaphase plate with the clearest chromosome configurations by calculating percent ratios of length for each chromosome to the total length of the haploid chromosomes (TCL). TCL is the total of lengths of all haploid autosomes and one sex chromosome (X or Z) when detected. These idiograms should be considered as tentative since good metaphase spreads were scarce and results are based on only one or a few chromosomal spread(s). Nevertheless, they served to obtain rough compositions of karyotypes. Classification of chromosomal



Figures 1-3.—Karyotypes of *Caddo agilis* and *Mitopus morio*: 1, *Caddo agilis*, female ( $2n = 30$ ); 2, 3, *Mitopus morio* ( $2n = 32$ ); 2, female; 3, male. Scale = 5  $\mu$ m.

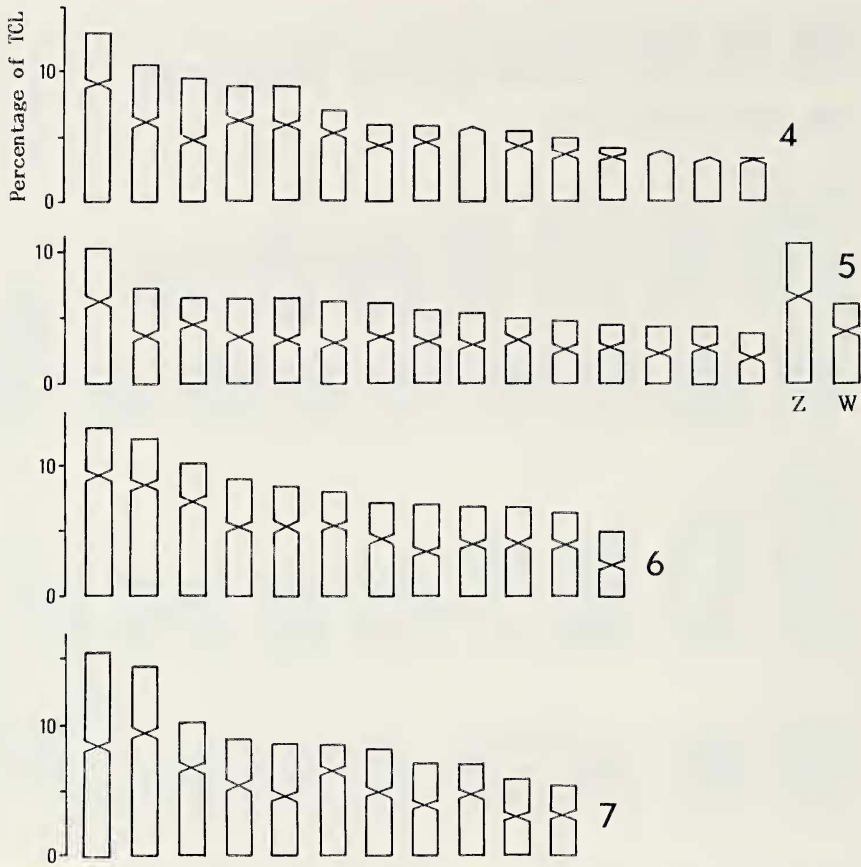
morphology was made according to Levans et al. (1964), where chromosomes are classified into the following five categories: metacentric ( $1.0 \leq r < 1.67$ ), submetacentric ( $1.67 \leq r < 3.0$ ), subtelocentric ( $3.0 \leq r < 7.0$ ), acrocentric ( $7.0 < r \leq \infty$ ) and telocentric, ( $r = \infty$ ).  $r = L/S$ , where L and S are lengths of long arm and short arm, respectively.

## RESULTS

### Family Caddidae

***Caddo agilis* Banks.**—  $2n$  (female) = 30 (Figs. 1, 4). Chromosomes were surveyed for females collected in 1982 from Nopporo, Hokkaido. A tentative idiogram based on some representative karyotypes (Fig. 1) is shown in Fig. 4. Chromosomes in which presence of short arm is unclear were prevalent in smaller ones; and chromosomes No. 9 or Nos. 13-15 were suggested to be telocentric or acrocentric. This species is considered to be parthenogenetic and only three males, one from North America and two from Japan, have been found (Gruber 1974; Suzuki and Tsurusaki 1983). The two males from Japan were collected in 1979 at Nopporo. However, no male has been found since then, so chromosomes of males of this species remain unknown.





Figures 4-7.—Idiograms of *Caddo agilis* and three species of Phalangiidae: 4, *Caddo agilis*, female; 5, *Mitopus morio*, female; 6, *Homolophus arcticus*, male; 7, *Dalquestia formosa*, male.

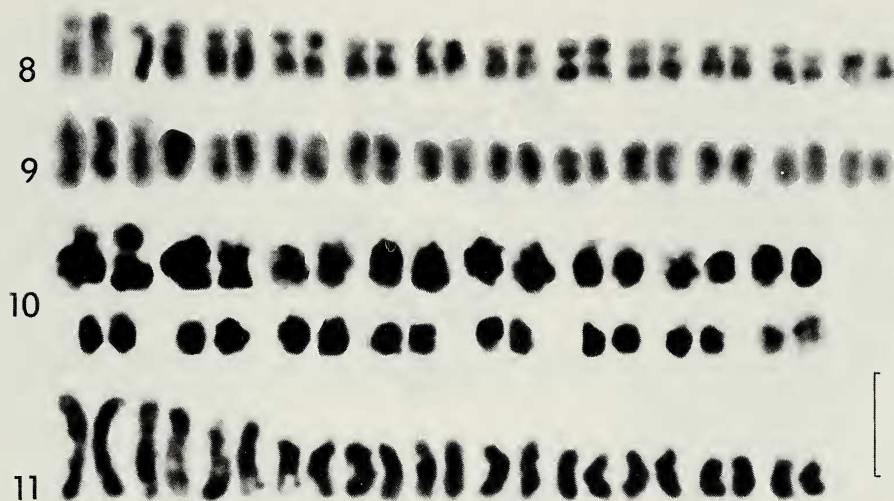
### Family Phalangiidae

#### Subfamily Phalangiinae

***Mitopus morio* Fabricius.**—  $2n$  (male, female) = 32 (Figs. 2-3, 5). This conforms to the number reported by Sokolow (1930) based on specimens from westernmost area of European part of U.S.S.R. and by Jennings (1982) on specimens from northern England. Only one cell from a female (Figs. 2, 5) provided a chromosomal spread acceptable for karyotype analysis. The karyotype seems to consist of 15 pairs of autosomes and one heteromorphic pair of chromosomes. Compared with a chromosome spread from the male (Fig. 3), chromosomes of this heteromorphic pair appeared to be sex chromosomes and correspond to Z and W chromosomes. Z chromosomes are the largest and metacentric, whereas W is metacentric and similar in size to chromosome No. 7. Autosomes are metacentric except for Nos. 3 and 12 which are submetacentric.

***Homolophus arcticus* Banks.**—  $2n$  (male) = 24 (Figs. 6, 8). No sex chromosomes were detected. The karyotype consisted of only metacentrics (Nos. 4, 5, 7-9, 12) and submetacentrics (others). In this respect, chromosome composition of this species is similar to that of *M. morio*.





Figures 8-11.—Karyotypes of males of four species of Phalangiidae: 8, *Homolophus arcticus* ( $2n = 24$ ); 9, *Homolophus rishiri* ( $2n = 24$ ); 10, *Phalangium opilio* ( $2n = 32$ ); 11, *Dalquestia formosa* ( $2n = 22$ ). Scale = 5  $\mu\text{m}$ .

***Homolophus rishiri* Tsurusaki.**—  $2n$  (male) = 24 (Figs. 9, 12). This  $2n$  number is the same as *H. arcticus*. Further analysis was not possible due to the indistinct chromosomal spread (Fig. 9). Numerous first meiotic metaphases showed 12 bivalents without exception (Fig. 12).

***Phalagium opilio* Linnaeus.**—  $2n$  (male) = 32 (Fig. 10). Only one spermatogonial metaphase plate, which is not enough for detailed karyotype analysis, could be found; it showed  $2n = 32$  clearly (Fig. 10). This number corresponds to that reported by Sokolow (1930) who studied the population in westernmost area of European U.S.S.R. However, this number does not agree with Juberthie (1956), who reported  $2n = 24$  for specimens from Moulis, Ariège, France. Further survey is needed to confirm whether this incongruence in chromosome number means a different species.

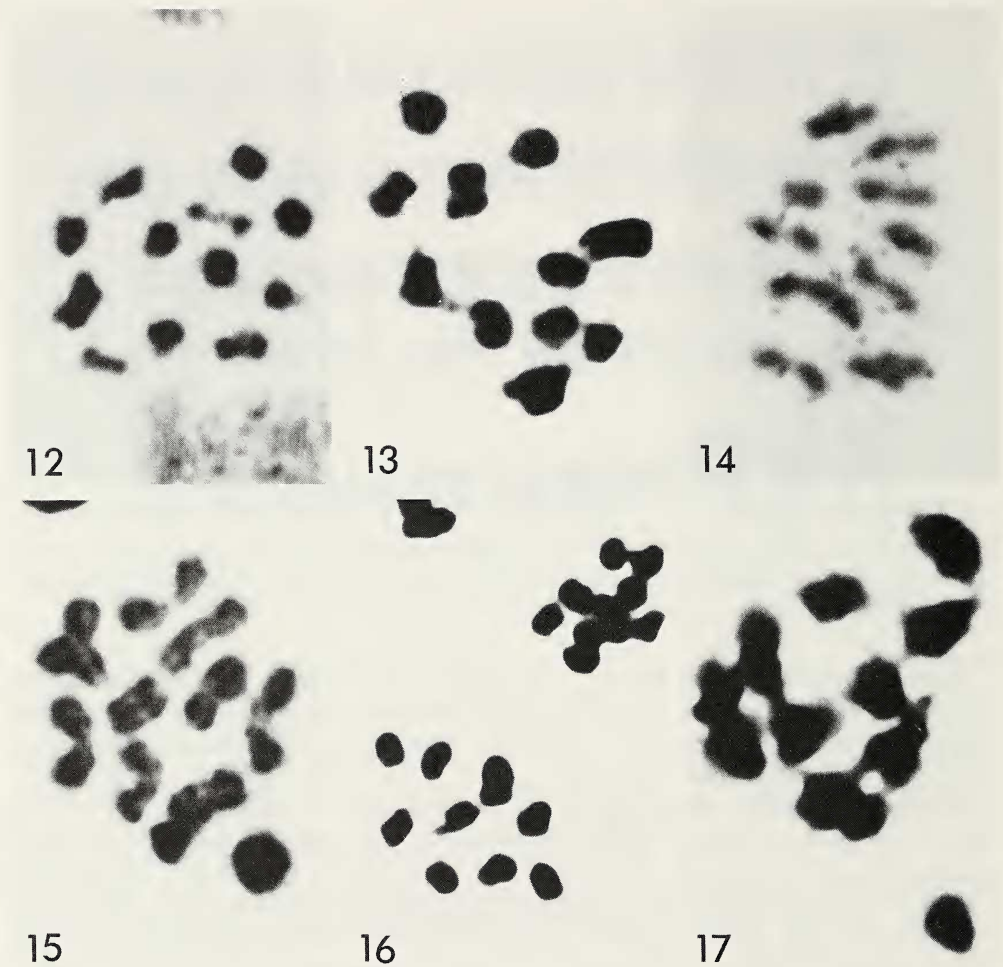
#### Subfamily unnamed

For comments on the placement of the genus *Dalquestia* Cokendolpher, see Cokendolpher (1984).

***Dalquestia formosa* (Banks).**—  $2n$  (male) = 22 (Figs. 7, 11). No sex chromosomes were detected. Karyotype consists of four pairs of submetacentrics (Nos. 2, 3, 6, 9) and seven pairs of metacentrics (others).

#### Subfamily Leiobuninae

***Nelima satoi* Suzuki.**—  $2n$  (male, female) = 16 (Figs. 18, 19, 25). The karyotype is composed of seven pairs of autosomes and one pair of male heterogametic sex chromosomes (male: XY, female: XX) (Figs. 18, 19). Autosomes are metacentric except for two pairs (Nos. 5, 7) being submetacentric



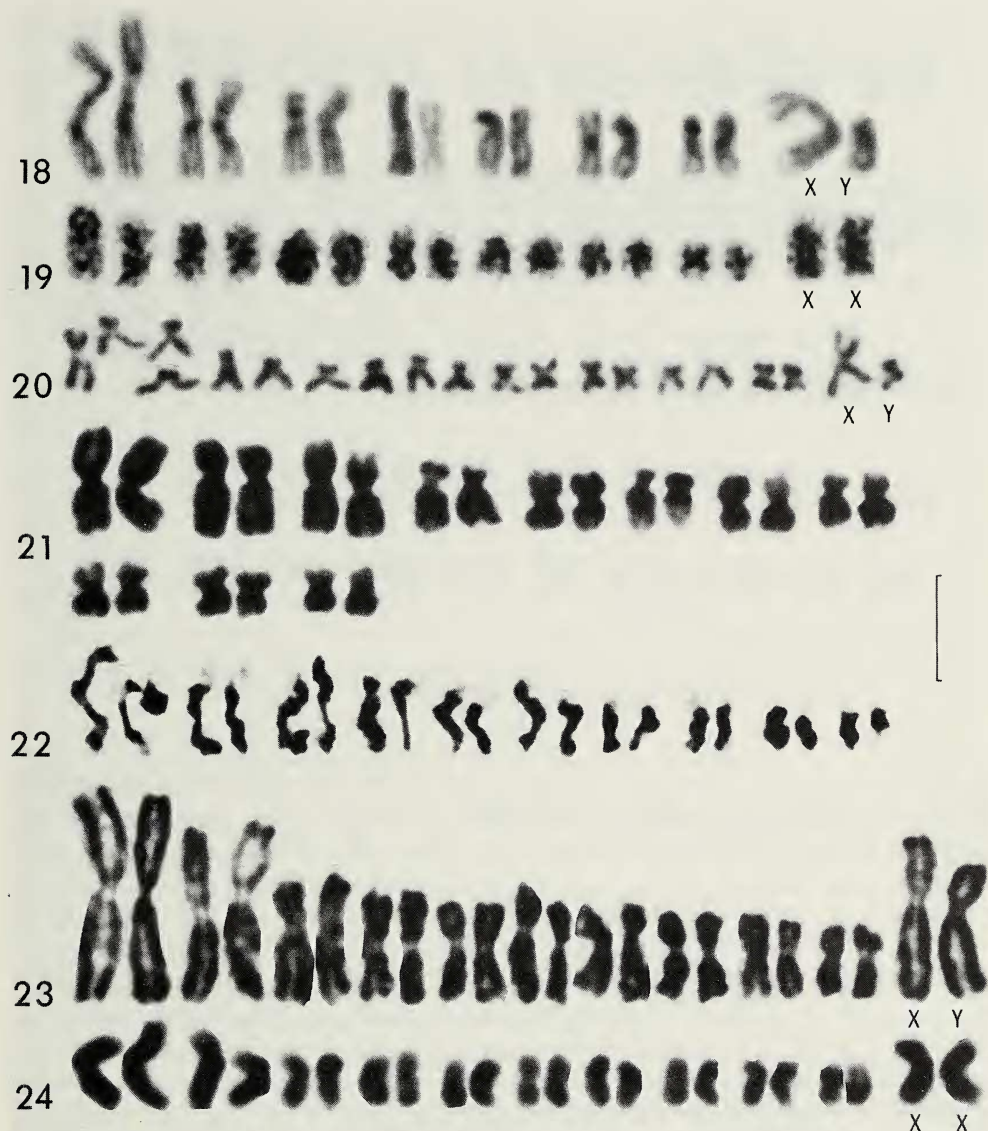
Figures 12-17.—Meiotic chromosomes in males: 12, *Homolophus rishiri*, metaphase I ( $n = 12$ ); 13, *Dalquestia formosa*, metaphase I ( $n = 11$ ); 14, *Melanopa grandis* (Mt. Ariake, Is. Tsushima), metaphase I ( $n = 10$ ); 15, 16, *Protolophus tuberculatus*; 15, metaphase I ( $n = 10$ ); 16, metaphase II ( $n = 9$ ); 17, *Protolophus* sp., metaphase I ( $n = 11$ ). Scale = 5  $\mu$ m.

(Fig. 25). The X chromosome is the second largest submetacentric, and Y is submetacentric similar in size to the shortest chromosome No. 7.

*Nelima similis* Suzuki.—  $2n$  (male) = 20 (Figs. 20, 26). The karyotype consisted of nine pairs of autosomes and one pair of heteromorphic sex chromosomes (Fig. 20). Autosomes are comprised of two pairs of submetacentrics (Nos. 2, 5) and seven pairs of metacentrics (others) (Fig. 26). The metacentric X and Y chromosomes are, respectively, the largest and the shortest.

*Leiobunum flavum* Banks.—  $2n$  (male) = 22 (Figs. 21, 27). The karyotype consisted of three pairs of submetacentrics (Nos. 4, 6, 8) and eight pairs of metacentrics (others). No sex chromosomes were discernible. This number,  $2n = 22$ , is the same as those reported in four species of *Leiobunum* C. L. Koch of North America (Parthasarathy and Goodnight 1958; Tsurusaki and Holmberg 1986).

*Leiobunum townsendi* Weed.—  $2n$  (male) = 20 (Fig. 22). Only one spermatogonial metaphase plate with 20 chromosomes was obtained (Fig. 22). Detailed karyotype is unknown.



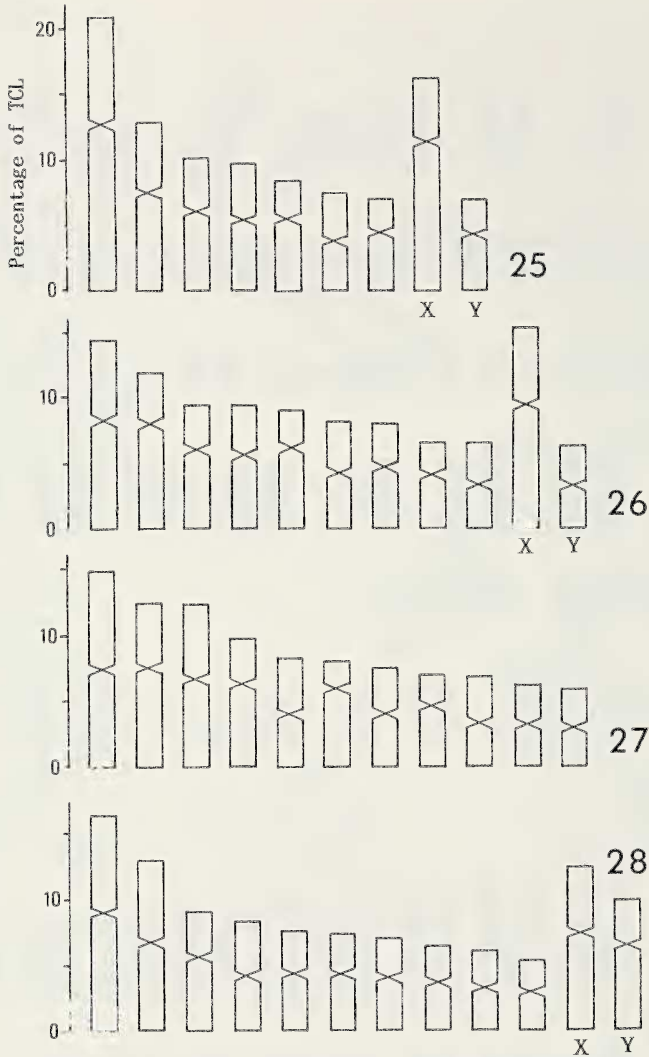
Figures 18-24.—Karyotypes of five species of Leiobuninae: 18, 19, *Nelima satoi* ( $2n = 16$ ); 18, male, Mt. Hiko; 19, female, Mt. Ishizuchi; 20, *Nelima similis* ( $2n = 20$ ), male; 21, *Leiobunum flavum* ( $2n = 22$ ), male; 22, *Leiobunum townsendi* ( $2n = 20$ ), male; 23, 24, *Eumesosoma roeweri* ( $2n = 22$ ); 23, male; 24, female. Scale = 5  $\mu$ m.

*Eumesosoma roeweri* (Goodnight and Goodnight).—  $2n$  (male, female) = 22 (Figs. 23, 24, 28). The autosomes were composed of ten pairs of metacentrics (Fig. 28). The X chromosome is a metacentric similar in size to chromosome No. 2; while Y is a submetacentric and somewhat smaller than X.

#### Subfamily Sclerosomatinae (?)

For the tentative placement of the genus *Protolophus* Banks, to which the following two species belong, in this subfamily, see Cokendolpher (1985). Large series of *Protolophus* spp. from various localities in the southwestern U.S.A.

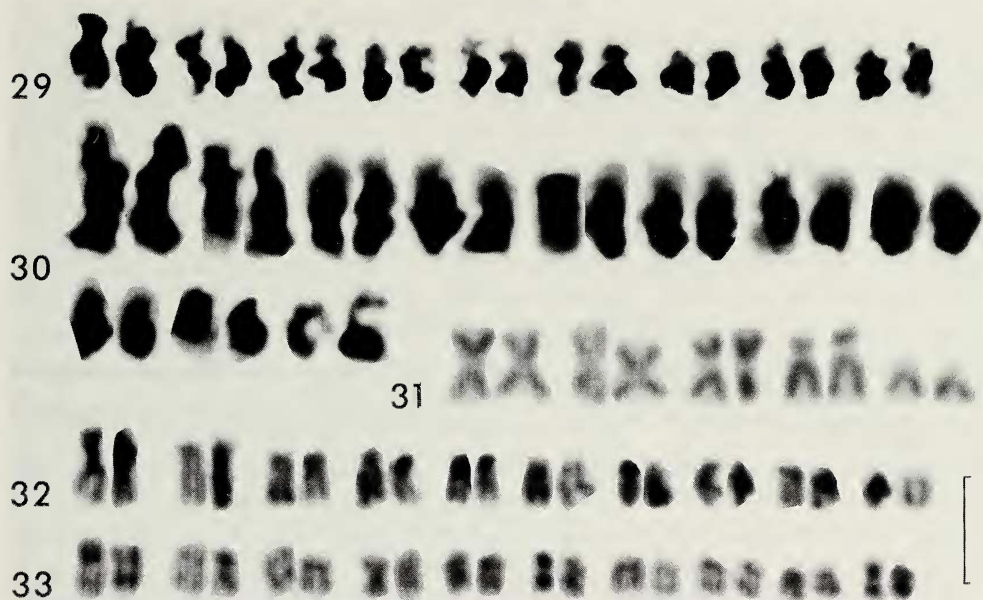




Figures 25-28.—Idiograms of males of four species of Leiobuninae: 25, *Nelima satoi*; 26, *Nelima similis*; 27, *Leiobunum flavum*; 28, *Eumesosoma roeweri*.

reveal the presence in many populations of two types of males: a larger, more robust type and a smaller, effeminate type. This type of dimorphism is rare in harvestmen. The differences in the pedipalps are dramatic, with normal males often having femora twice as thick as those of effeminate males of the same population. One of us (J.C.C.) has thought for many years that these differences were due to a different number of molts for the two forms to reach adulthood. Attempts to rear *Protolophus* spp. in the laboratory (by J.C.C.) have failed, but successful copulations have been observed between single females and both types of males.

***Protolophus tuberculatus* Banks.**—  $2n$  (male) = 18 and 20 (Figs. 15, 16, 29). Two males (one normal, one effeminate) collected from the same locality in San Anselmo, California but in different years, respectively 1983 and 1984, were used for chromosome observation. The result reveals the two forms have different chromosome numbers. That is, the effeminate male collected in the summer of



Figures 29-33.—Karyotypes of males of four species of Sclerosomatinae and Gagrellinae: 29, *Protolophus tuberculatus* ( $2n = 18$ ); 30, *Protolophus* sp. ( $2n = 22$ ); 31, *Trachyrhinus rectipalpus* ( $2n = 10$ ); 32-33, *Melanopa grandis* ( $2n = 20$ ); 32, Lake Misuzu; 33, Hidakatsu, Is. Tsushima. Scale = 5  $\mu$ m.

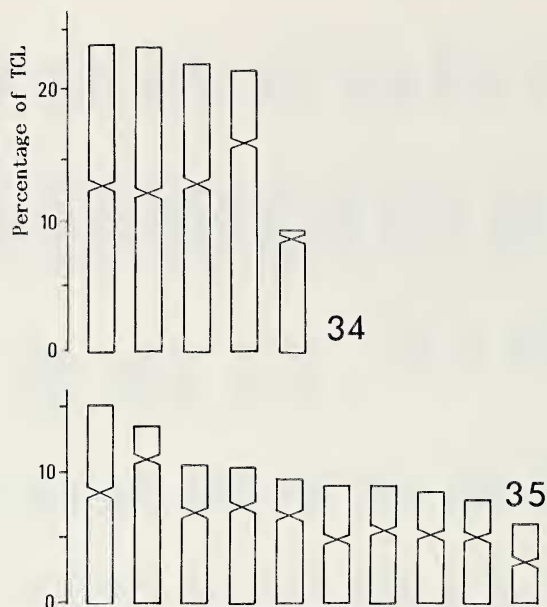
1983 showed  $n = 10$  (hence it is expected to be  $2n = 20$ ) in its first and second meiotic metaphase plates (Fig. 15), whereas the normal male from sampling in 1984 showed chromosome number  $2n = 18$  and  $n = 9$  (Figs. 29 and 16). Detailed karyotype of the latter is unknown, although most of the chromosomes seem to be submeta- or metacentric. Further study, including females, is needed to understand the implication of this discrepancy in chromosome number.

***Protolophus* sp.**—  $2n$  (male) = 22 (Fig. 30). Only one spermatogonial metaphase spread with 22 chromosomes was obtained. Detailed composition of chromosomes is unknown, although most chromosomes seem to be metacentric or submetacentric (Fig. 30). Since this species could not be identified, we are depositing the specimen in the collection of the California Academy of Sciences, San Francisco.

#### Subfamily Gagrellinae

***Trachyrhinus rectipalpus* Cokendolpher.**—  $2n$  (male) = 10 (Figs. 31, 34). The karyotype consists of three pairs of metacentric (Nos. 1-3), one pair of submetacentric (No. 4), and one pair of small acrocentric chromosomes (Figs. 31 and 34). No sex chromosomes were detected. This chromosome number,  $2n = 10$ , is the lowest reported in Opiliones, ranking with *Systenocentrus japonicus* Hirst and *Paraumbogrella pumilio* (Karsch) (Tsurusaki 1982; also see below).

***Melanopa grandis* Roewer.**—  $2n$  (male) = 20 (Figs. 32, 33, 35). Chromosomes were surveyed for specimens from six localities which represent three different geographic forms defined as follows in terms of structure of male palpi (P) and female genital operculum (GO) (cf. Suzuki 1972).



Figures 34-35.—Idiograms of males of two species of Gagrellinae: 34, *Trachyrhinus rectipalpus*; 35, *Melanopa grandis*.

*Form I*: male with normal but robust P and female with three (sometimes two) -sectioned GO [figs. 1(6-8) and 3IJK].

*Form II*: male with robust P having trigger-shaped tibiae; female with two-sectioned GO [figs. 1(9) and 3H].

*Form III*: male with normal and slender P; female with unsectioned GO [figs. 1(5) and 3E in Suzuki, 1972].

In spite of the prominent geographic variation in external morphology, numbers of chromosomes were determined to be  $2n = 20$  ( $n = 10$ ) without exception. Chromosomes of this species were generally so small in size ( $2.2 \mu\text{m}$  on average) that few chromosome spreads could be analyzed in detail. Of these, representative karyotypes from Lake Misuzu, Nagano Pref. (Form I) and Hidakatsu on Is. Tsushima (Form III), and an idiogram based on the former are shown in Figs. 32, 33 and 35, respectively. The karyotype consisted of five pairs of metacentrics (Nos. 1, 6-8, 10), four pairs of submetacentrics (Nos. 3-5, 9), and one pair of subtelocentrics (No. 2). No sex chromosomes were detected.

*Paraumbogrella pumilio* (Karsch).—  $2n$  (male) = 10. On the basis of specimens from Sapporo, Hokkaido, Tsurusaki (1982) reported chromosomes of this species as  $2n$  (male, female) = 10 and XY (male) - XX (female) in its sex chromosome constitution under the name *P. huzitai* Suzuki (see Suzuki 1985, for the name change). This time, a male collected from Sunagawa, which is located about 70 km northeast of Sapporo, was chromosomally examined. Although no chromosome spreads sufficient for analysis could be obtained, chromosome number was clearly counted as  $2n = 10$ .



## DISCUSSION

Table 2 is a compilation of the number of chromosomes and sex chromosome system so far recorded of various opilionid species, belonging to Caddidae and Phalangiidae. A comparison at subfamilial level reveals that chromosome numbers tend to be greater in Caddinae, Caddidae ( $2n = 30$ ) or Phalangiinae ( $2n = 20-36$ ), fewer in Gagrellinae ( $2n = 10-22$ ), and intermediate in Leiobuninae ( $2n = 16-26$ ).

However, chromosome number often fluctuates within the genus, sometimes even within a species (e.g., *Leiobunum montanum* Suzuki: Tsurusaki 1985b). This forms a contrast with the situation in most spiders where the chromosome numbers are relatively stable at the familial level (Hackman 1948; Suzuki 1954; Datta and Chatterjee 1983). Difference in population structure between both groups of animals may partly explain this disparity. That is, probability that newly emerged chromosomal variants are fixed in a population may be relatively high in opilionids due to their low vagility which promotes inbreeding and drift. On the other hand, in spiders, inbreeding and drift would be unlikely to occur, since ballooning would facilitate both dispersal of the sibs and gene flow among populations. Consequently, even if a chromosomal mutation did occur within a population of spiders, the prospect that this mutant would predominate existent chromosomes would be low. Thus, karyotype evolution in spiders is expected to be conservative. Such correlation between population structuring and evolutionary rate of karyotypic evolution is found in various animal groups and is also theoretically supported (White 1978; Bush 1981).

On the other hand, in spite of great diversity in number of chromosomes, both meta- and submetacentrics overwhelmingly predominate in the component chromosomes of Opiliones, compared to telo- or acrocentrics (Figs. 4-7, 25-28, 34, 35; cf. also Tsurusaki 1985b). This fact suggests that Robertsonian translocation is not a main cause for the change of chromosome number. Further, this also makes a contrast with the situation in spiders where chromosomes are usually structured as telo- or acrocentrics (Hackman 1948; Suzuki 1954; Kageyama et al. 1978; Kageyama and Seto 1979). Primary factors for the difference in chromosome structure between the two groups are still incompletely known.

Sex chromosome composition in Opiliones has been determined as usually XY-XX (male heterogametic) based on *Paraumbogrella pumilio* and some species of *Leiobunum* (Tsurusaki 1982, 1985a, b; Tsurusaki and Holmberg 1986). In addition to these species, *Nelima satoi*, *N. similis*, and *Eumesosoma roeweri* were also revealed to have the same system of sex chromosomes in the present study. On the other hand, presence of female heterogamety with ZW (female) - ZZ (male) was suggested in *Mitopus morio*. It deserves attention, since no species with female heterogamety has hitherto been recorded in arachnids (White 1973; Bull 1983: 17). There is a possibility that this sex chromosome system predominates in species of Phalangiinae, since (1) we failed to detect any heteromorphic sex chromosomes in males of the other species of Phalangiinae examined in this work and (2) female heterogamety is also suggested in *Oligolophus aspersus* (Karsch), one of the relatives of *M. morio* (N. T. unpubl.). Further survey using material of both sexes of various species is needed. Other

Table 2.—Number of chromosomes and sex determination in various species of opilionids belonging to families Caddidae and Phalangiidae. M = male, F = female. 2n chromosome number in parentheses denotes the one inferred from haploid number alone. References are abbreviated as follows: 1, Jennings (1982); 2, Juberthie (1956); 3, Parthasarathy and Goodnight (1958); 4, Sharma and Dutta (1959); 5, Sokolow (1930); 6-12, Suzuki (1941, 1957, 1966, 1976a, 1976b, 1980, 1986); 13, Tomohiro (1940); 14-16, Tsurusaki (1982, 1985a, 1985b); 17, Tsurusaki and Holmberg (1986); NT, Tsurusaki unpubl.; PS, Present study.

Species	Locality	Sex	2n chrom. number	Type of sex determ.	Refer.
Family Caddidae					
<i>Caddo agilis</i> Banks	Japan: Hokkaido, Nopporo	F	30	—	PS
Family Phalangiidae					
Subfamily Phalangiinae					
<i>Oligolophus aspersus</i> (Karsch)	Japan: various localities	M,F	20	ZW(?)	6, NT
<i>Oligolophus tridens</i> (C. L. Koch)	U.S.S.R.: Leningrad	M	32	—	5
<i>Mitopus morio</i> (Fabricius)	U.S.S.R.: Leningrad	M	32	—	5
	England: northern part	M	32	—	1
	Japan: Is. Rishiri	M,F	32	ZW	PS
<i>Mitopus ericaeus</i> Jennings	England: northern part	M	32	—	1
<i>Opilio parientinus</i> (De Geer)	U.S.S.R.: Leningrad	M	24	—	5
<i>Homolophus arcticus</i> Banks	Japan: Hokkaido	M	24	—	PS
<i>Homolophus rishiri</i> Tsurusaki	Japan: Is. Rishiri	M	24	—	PS
<i>Phalangium opilio</i> Linnaeus	U.S.S.R.: Leningrad	M	(32)	—	5
	France	M	24	—	2
	U.S.A.: Idaho, Moscow	M	32	—	PS
<i>Rilaena triangularis</i> (Herbst) (= <i>Platybunus triangularis</i> : in ref. 5)	U.S.S.R.: Leningrad	M	(36)	—	5
Subfamily unnamed					
<i>Dalquestia formosa</i> (Banks)	U.S.A.: Texas	M	22	—	PS
Subfamily Leiobuninae					
<i>Nelima satoi</i> Suzuki	Japan	M,F	16	XY	PS
<i>Nelima similis</i> Suzuki	Japan: Nagano Pref.	M	20	XY	PS
<i>Leiobunum japonense</i> <i>japonicum</i> (Suzuki)	Japan	M	16	—	10

<i>Leiobunum japonicum japonicum</i> Müller	Japan	M,F	20	XY	6,17
<i>Leiobunum paessleri</i> Roewer	Canada: British Columbia	M	22	XY	17
<i>Leiobunum crassipalpe</i> Banks	U.S.A.: details unknown	M	22	—	3
<i>Leiobunum nigripes</i> Weed	U.S.A.: details unknown	M	22	—	3
<i>Leiobunum ventricosum</i> Wood	U.S.A.: details unknown	M	22(?)	—	3
<i>Leiobunum flavum</i> Banks	U.S.A.: Texas	M	22	—	PS
<i>Leiobunum townsendi</i> Weed	U.S.A.: Texas	M	20	—	PS
<i>Leiobunum rupestre</i> (Herbst)	U.S.S.R.: Leningrad	M	22	—	5
<i>Leiobunum hikocola</i> Suzuki	Japan: Kyushu, Mt. Hiko	M	18	XY	15
<i>Leiobunum montanum</i> Suzuki	Japan: various localities	M,F	18-26	XY	9,16
<i>Leiobunum hiasai</i> Suzuki	Japan: Yamanashi Pref.	M	(24)	—	15
<i>Leiobunum sadoense</i> Tsurusaki	Japan: Is. Sado	M	(18)	—	15
<i>Leiobunum kohyai</i> Suzuki	Japan: Honshu	M	20	XY	9,15
<i>Leiobunum hiraiwai</i> (Sato and Suzuki)	Japan: various localities	M,F	18-22	XY	7,11,NT
<i>Leiobunum curvipalpe</i> Roewer	Japan: various localities	M,F	24	XY	7,NT
<i>Eumesosoma roeweri</i> (Goodnight and Goodnight)	U.S.A.: Texas	M,F	22	XY	PS
Subfamily Sclerosomatinae (?)					
<i>Protolophus tuberculatus</i> Banks	U.S.A.: California	M	18,(20)	—	PS
<i>Protolophus</i> sp.	U.S.A.: California	M	22	—	PS
Subfamily Gagrellinae					
<i>Trachyrhinus rectipalpus</i> Cokendolpher	U.S.A.: Texas	M	10	—	PS
<i>Gagrellopsis nodulifera</i> Sato and Suzuki	Japan: Hiroshima Pref.	M	16	—	13
<i>Gagrellula ferruginea</i> (Loman)	Japan: various localities	M,F	10-22	—	6,12,NT
<i>Melanopa grandis</i> Roewer	Japan: various localities	M	20	—	PS



<i>Melanopa unicolor</i> Roewer	India	M	18	—	4
<i>Systemocentrus</i> <i>japonicus</i> Hirst	Japan	M	(10)	—	8
<i>Paraumbogrella</i> <i>pumilio</i> (Karsch)	Japan; Hokkaido	M	10	XY	14, PS

than these, Parthasarathy and Goodnight (1958) suggested the presence of XO-XX (male heterogametic) system in opilionids based on their observation on *Vonones sayi* (Simon) (= *V. ornata*: in their paper) of family Cosmetidae (suborder Laniatores). This statement is somewhat dubious, however, since diploid number of chromosomes of this species may not be 25 as they reported but far more numerous [probably  $2n = 78$  (male, female): J.C.C. pers. obs.]. Nevertheless, the possibility that XO system also will be found in other opilionids cannot be excluded. The XO type and its derivatives (XXO, XXXO, etc.) are ordinary systems in ticks (Oliver 1981) and particularly in Araneae where these systems are exclusive (Hackman 1948; Suzuki 1954) except for four species of the salticid genus *Pellenes* Simon having  $X_1X_2X_3Y$  male,  $X_1X_1X_2X_2X_3X_3$  female system (Maddison 1982) and some populations of huntsman spider, *Delena cancerides* Walckenaer having a kind of multiple XY sex-determining mechanism (Rowell 1985).

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## APPENDIX

**Collecting data of the materials.**—These are given by the following order: Locality, date collected (Unless the materials are dissected on the same day or day after, dates of fixation is also given in parentheses), collector (N. T. = N. Tsurusaki, J. C. C. = J. C. Cokendolpher), number of individuals (Number in parentheses denotes the number of specimens dissected. This number may be unequal to the one in Table 1, since there were several slides that contained no countable chromosomal spreads).

1. *Caddo agilis*. JAPAN: HOKKAIDO; Ebetsu; Nopporo, 18 June 1982 (N. T.), 6 females; same locality, 21 June 1982 (N. T.), 3 females.
2. *Mitopus morio*. JAPAN: HOKKAIDO; Is. Rishiri; Mt. Rishiri, From Oshidomari to Pon-yama, 30-320 m alt., 8 July 1984 (N. T.), 1 male, 5 juveniles (5 juveniles).
3. *Homolophus arcticus*. JAPAN: HOKKAIDO; Teshio-gun; Toyotomi-chô; Wakasakanai, 9 August 1985 (N. T.), 3 males, 1 female, 16 juveniles (10 juveniles).
4. *Homolophus rishiri*. JAPAN: HOKKAIDO; Is. Rishiri; Mt. Rishiri; Oshidomari route, 670-1000 m alt., 8 August 1985 (N. T.), 2 males, 1 female, 3 juveniles (3 juveniles).
5. *Phalangium opilio*. U.S.A.: IDAHO; Latan Co.; Moscow, 14 September 1983 (F. W. Merickel), 1 male.
6. *Dalquestia formosa*. U.S.A.: TEXAS; Kerr Co.; 3.2 km SSE Center Point. 16 September 1983 (W. Rogers), 1 male.
7. *Nelima satoi*. JAPAN: EHIME PREF.; Mt. Ishizuchi, From Tsuchigoya to Mt. Iwaguro, 1490-1745 m alt., 5 August 1982 (N. T.), 2 juveniles.
8. *Nelima similis*. JAPAN: NAGANO PREF.; Kami-Ina-gun; Takatô, Hokomochi Shrine, 780 m alt., 20 August 1982 (N. T.), 16 males, 7 females, 6 juveniles (4 males).
9. *Leiobunum flavum*. U.S.A.: TEXAS; Walker Co.; Sam Houston National Forest, Lake Stubblefield, 29 August 1984 (S. W. Taber), 6 males.
10. *Leiobunum townsendi*. U.S.A.: TEXAS; Concho Co.; Colorado River crossing at Highway 2134 (31°34'N - 99°41'W), 11 June 1983 (fixed 5 August 1983) (F. L. Rose, L. Robbins and K. W. Selcer), 1 male.
11. *Eumesosoma roeweri*. TEXAS: Concho Co.; Colorado River crossing at Highway 2134 (31°34'N - 99°41'W), 11 June 1983 (F. L. Rose, L. Robbins and K. W. Selcer), 2 males, 2 females; Kerr Co.; 6.4 km E of Kerrville, 17 May 1984 (S. R. Jones), 3 males, 1 female.
12. *Protolophus tuberculatus*. U.S.A.: CALIFORNIA; Marin Co.; San Rafael Ridge at 800 Fawn Drive, San Anselmo, 15 May 1983 (fixed 22 May 1983) (L. G. Frehofer), 1 male; same locality, 19 March 1984 (fixed 4 April 1984) (L. G. Frehofer), 1 male.
13. *Protolophus* sp. U.S.A.: CALIFORNIA; Ventura Co.; Little Sycamore Canyon, ca. 1.6 km N Pacific Coast Highway (35°5'N - 118°57'W), 28 June 1985 (fixed 1 July 1985) (J. C. C.), 2 males.
14. *Trachyrhinus rectipalpus*. U.S.A.: TEXAS; McMullen Co.; 36.8 km S of Tilden, 20 May 1985 (fixed 24 May 1985) (S. W. Taber), 1 male.
15. *Melanopa grandis*. JAPAN: NAGANO PREF.; Matsumoto; Lake Misuzu, 980 m alt., 29 June 1984 (fixed 5 July 1984) (N. T.), 1 juvenile; Mt. Kirigamine, Kowashimizu campground, 1630 m alt., 8 July 1982 (N. T.), 2 juveniles (1 juvenile).
- TOTTORI PREF.; Mt. Daisen, 760-1100 m alt., 9 August 1982 (N. T.), 14 males, 9 females (2 males).
- FUKUOKA PREF.; Mt. Hiko, 640-800 m alt., 31 July 1982 (N.T.), 7 males, 6 females (3 males).
- NAGASAKI PREF.; Is. Tsushima; Kamitsusima-chô, Hidakatsu, 50-60 m alt., 26 July 1982 (N. T.), 17 males, 18 females (3 males); Is. Tsushima; Izuhara, Mt. Ariake, 200-530 m alt., 27 July 1982 (N. T.), 9 males, 5 females (3 males).
16. *Paraumbogrella pumilio*. JAPAN: HOKKAIDO; Sunagawa, on a levee of River Penke-Utashinai, near the city hall, ca. 25 m alt., 24 September 1986 (N. T.), 1 female; same locality, 1 October 1986 (N. T.), 1 male, 1 female.